Accepted Manuscript

Rational Design, Synthesis, and Structure–Activity Relationships of 5-Amino-1H-pyrazole-4-carboxylic acid derivatives as protein tyrosine phosphatase 1B inhibitors

Sujay Basu, Philip Prathipati, Sachin Thorat, Shariq Ansari, Meena Patel, Vaibhav Jain, Ramana R Jinugu, Sanjay Niranjan, Siddhartha De, Satyanarayana Reddy

PII: S0968-0896(16)30927-0
DOI: http://dx.doi.org/10.1016/j.bmc.2016.10.012
Reference: BMC 13339

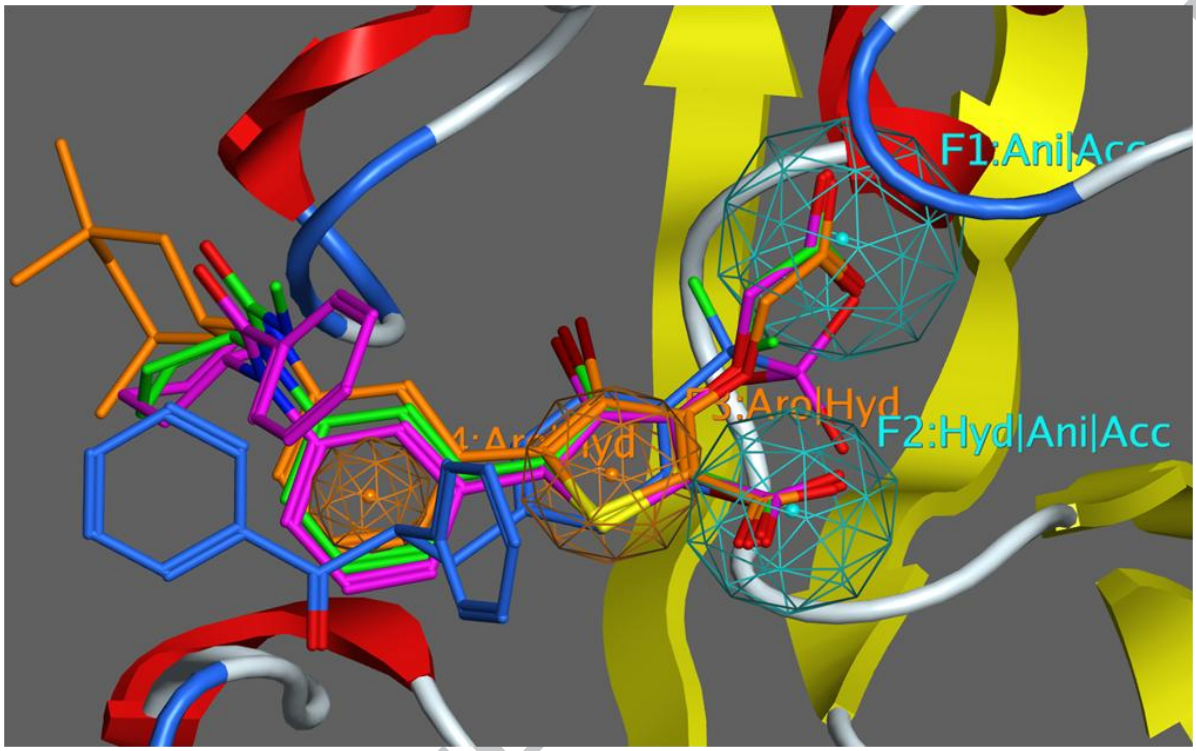
To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 8 August 2016
Revised Date: 5 October 2016
Accepted Date: 8 October 2016

Please cite this article as: Basu, S., Prathipati, P., Thorat, S., Ansari, S., Patel, M., Jain, V., Jinugu, R.R., Niranjan, S., De, S., Reddy, S., Rational Design, Synthesis, and Structure–Activity Relationships of 5-Amino-1H-pyrazole-4-carboxylic acid derivatives as protein tyrosine phosphatase 1B inhibitors, *Bioorganic & Medicinal Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.10.012

Graphical abstract

.



Rational Design, Synthesis, and Structure–Activity Relationships of 5-Amino-1H-pyrazole-4-carboxylic acid derivatives as protein tyrosine phosphatase 1B inhibitors

Sujay Basu*, Philip Prathipati, Sachin Thorat, Shariq Ansari, Meena Patel, Vaibhav Jain, Ramana R Jinugu, Sanjay Niranjan, Siddhartha De, Satyanarayana Reddy.

Drug Discovery Facility, Advinus Therapeutics Ltd, Quantum Towers, Plot-9, Phase-I, MIDC, Hinjewadi, Pune 411 057, India.

Abstract

A series of novel amino-carboxylic based pyrazole as protein tyrosine phosphatase 1B (PTP1B) inhibitors were designed on the basis of structure-based pharmacophore model and molecular docking. Compounds containing different hydrophobic tail (1,2-diphenyl ethanone, oxdiadizole and dibenzyl amines) were synthesized and evaluated in PTP1B enzymatic assay. Structure–activity relationship based optimization resulted in identification of several potent, metabolically stable and cell permeable PTP1B inhibitors.

***Keywords**:* Protein Tyrosine Phosphatase 1B; Pharmacophore; Permeability; Hydrophobic; Oxadiazole.

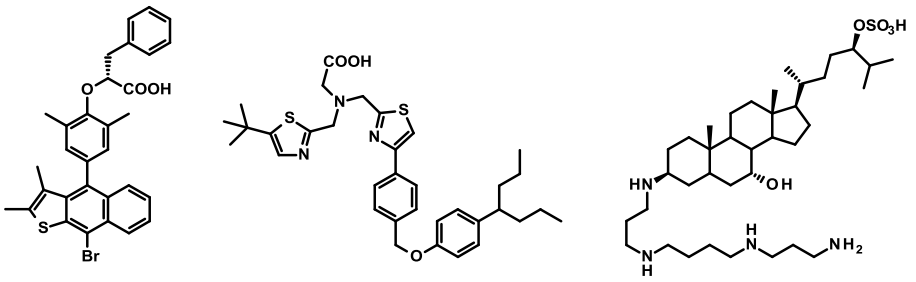
* Corresponding author. Tel.: +91 20 66539600; fax: +91 20 66539620.

E-mail address: sujay.basu@advinus.com (B, Sujay)

1. Introduction

Protein tyrosine phosphatase 1B (PTP1B) is an intracellular protein expressed in insulin responsive tissues including the classical insulin targeted tissues such as liver, muscle and fat.[1] PTP1B plays an important role in insulin receptor signaling.[2] PTP1B dephosphorylates the insulin receptor during its biosynthesis in endoplasmic reticulum as well as after it has been stimulated by the insulin, and thus play a central role in negative regulation of insulin signaling pathway.[3] Out of several negative regulators, PTP1B has gained importance because of its specificity and *in vivo* validation in animal models of diabetes.

The discovery of potent, selective, cell permeable and orally bioavailable PTP1B inhibitors is a challenging medicinal chemistry objective. PTP1B inhibitors need to have good cellular penetration, as target is intracellular, and orally available drug is desired. This is formidable task because of the physical nature of this target. Most of the small PTP1B inhibitors bear highly charged phosphonates[4] or multiple acid and peptide functionalities[5] due to which they show poor cell permeability and oral bioavailability. Out of several small molecule PTP1B inhibitors, only three small molecule PTP1B inhibitors entered clinical trials (Figure 1) and finally discontinued due to insufficient efficacy and unwanted side effects. In our earlier communications we have reported cell permeable and orally bio-available PTP inhibitors. The journey was started with designing of "heterocyclic based carboxylic acids" that resembles as "head group" and followed by synthesis of the compounds containing "hydrophobic tail".[6]



Ertiprotafib **JTT-551** **Trodusquemine**

Figure 1. PTP1B inhibitors reached clinical trials.

2. Inhibitor Design

Multiple chemotypes and their co-crystal structures are known in the literature for PTP1B inhibitors. We have shortlisted (Figure 2) different head group pharmacophores which have been successfully used to design PTP1B inhibitors.[7]

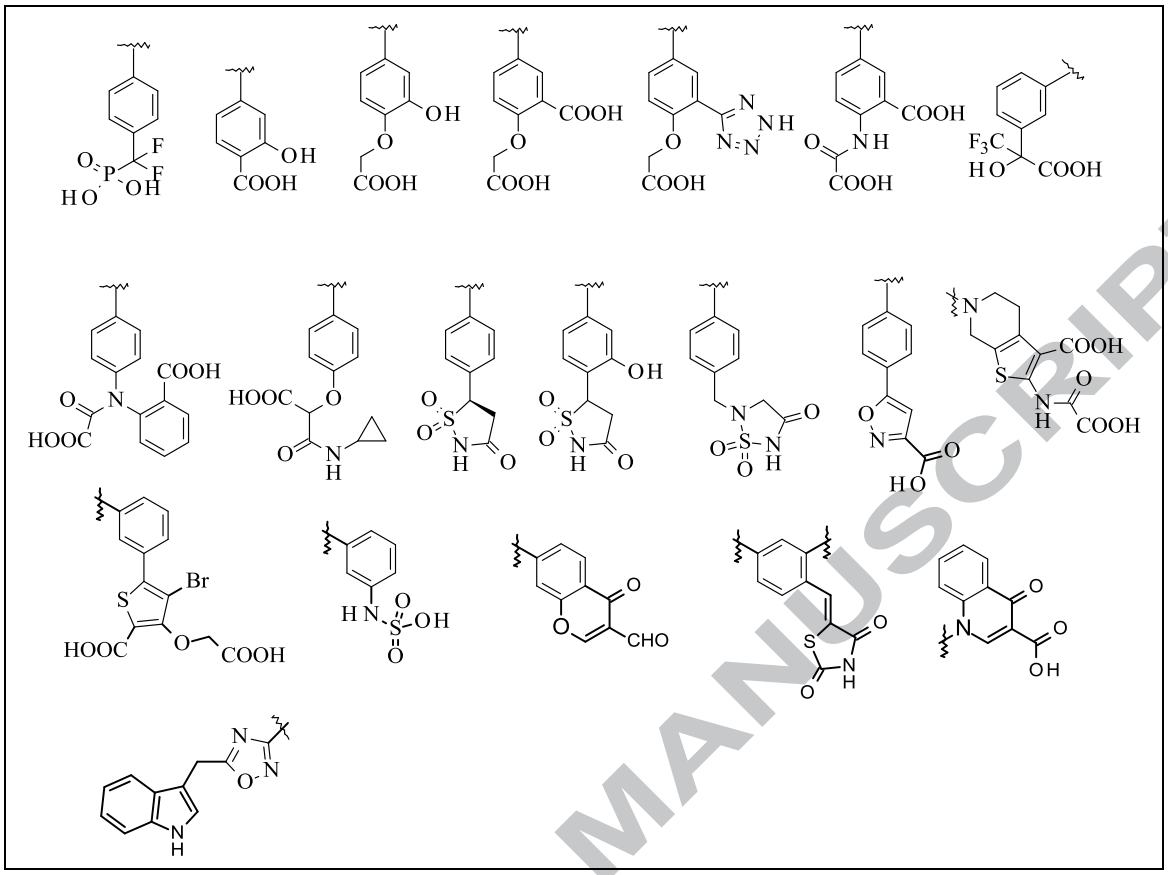
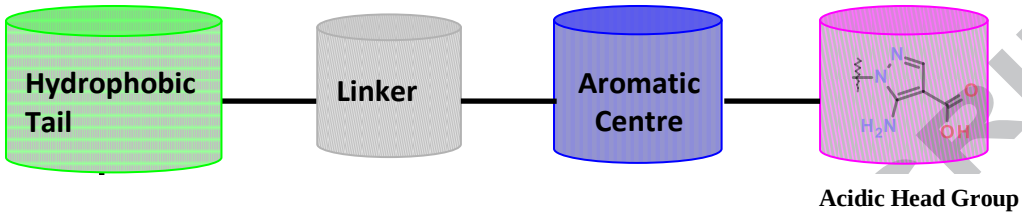


Figure 2. Literature reported PTP1B inhibitors acidic head group pharmacophore.

The active site of PTP1B has evolved to accommodate phosphotyrosine (*p*-Tyr) residue which contains two negative charges at physiological pH. Hence the inhibitors developed against PTP1B were also having negative charges to compete with *p*-Tyr. But these inhibitors are also likely to have negative impact on cell permeability. One plausible way to counter this problem is to increase pKa of terminal –COOH and therefore increase the concentration of the neutral form in physiological environment.[8-10] We envisaged to design ligands by introduction of new pharmacophore, $–NH_2$ group next to –COOH group in order to increase pKa of the –COOH (Figure 3). A typical structural design of PTP1B inhibitors is shown in Figure 2, which comprises of amino pyrazole carboxylic acid based "head group", an aromatic centre and a hydrophobic "aryl tail" with a spacer in-between. We have also calculated pKa (using

ChemAxon, http://www.chemaxon.com) of designed compounds and a few examples are given in Table 1. It was found that pKa of –COOH increased after introduction of $–NH_2$ which may help to improve cell permeability.



Examples of hydrophobic tail

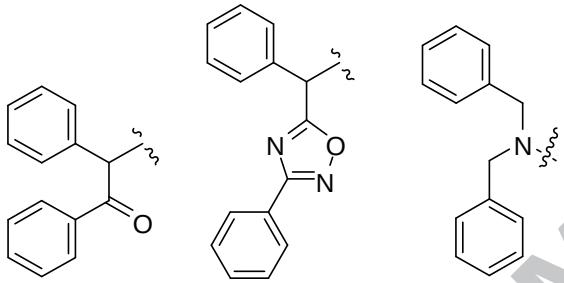
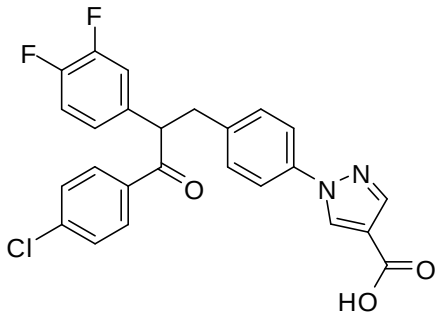
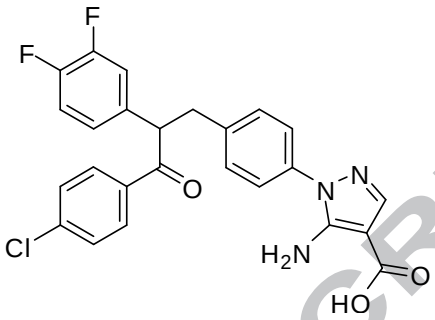
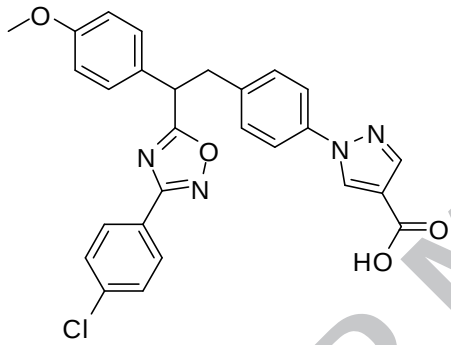
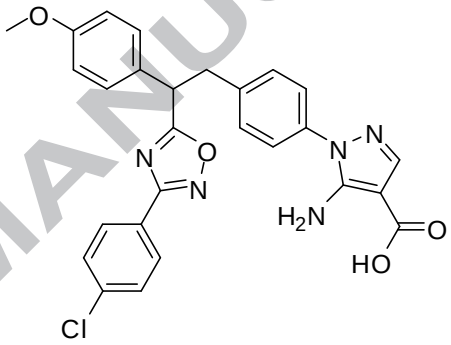
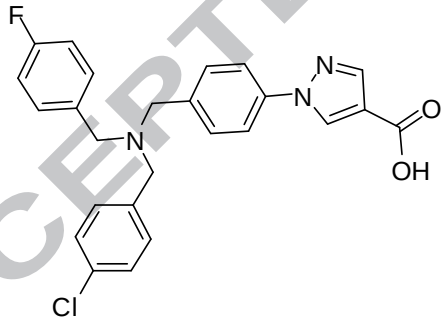
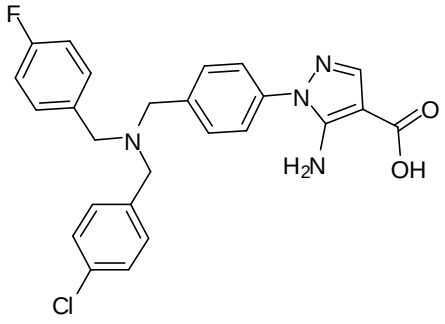


Figure 3. Amino pyrazole containing carboxylic acid as head group.

Table 1. pKa of representative designed ligands.

pKa of –COOH= 3.42	pKa of –COOH= 4.11 (**8j**)
	
pKa of –COOH= 3.41	pKa of –COOH= 4.01 (**11f**)
	
pKa of –COOH= 3.38	pKa of –COOH= 4.07 (**16c**)
	

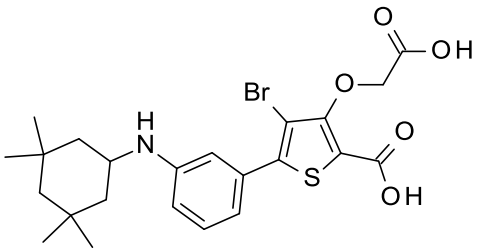
2.1. Structure-based 3D Pharmacophore model and molecular docking

3D Pharmacophore model:

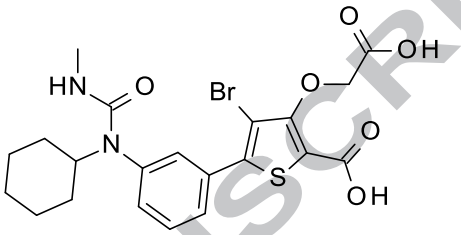
The purpose of generating the pharmacophore model was to identify essential features/functional groups in the PTB1B inhibitors, which are responsible for the inhibitory activity. Moreover, it

was also used as a tool to validate the designed ligands. An integrated ligand and structure-based pharmacophore model was developed for PTP1B inhibitors using PharmaGist and MOE (consensus pharmacophore using PCH scheme- Polar, charged, hydrophobic), respectively.

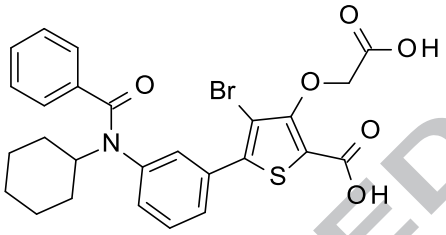
2QBQ (PTP1B, K_i : 36 nM)



2ZMM (PTP1B, K_i : 25 nM)



2ZN7(PTP1B, K_i : 13 nM)



3CWE (PTP1B, K_i : 120 nM)

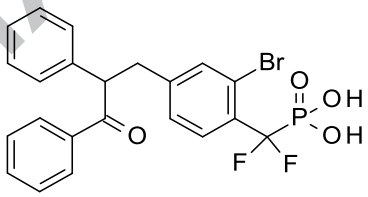


Figure 4. Known PTP1B co-crystallized ligands.

The generated pharmacophoric hypothesis was derived using the known PTP1B co-crystallized ligands (PDB ID: 4B3-2QBQ; 35B-2ZMM; 410-2ZN7; 825-3CWE, Figure 4) as templates. The structure-based pharmacophore model (Figure 5) consisted of four pharmacophoric features; two charged features (negative ionizable/hydrogen bond acceptor, Features 1 & 2), two aromatic/hydrophobic features (Features 3 & 4). The interactions captured by each of the pharmacophoric features with PTP1B active site is depicted in Figure 5. The pharmacophore analysis revealed that the difluoromethylene phosphonate (DFMP) of 3CWE or 3-thienyloxy acetic acid (2QBQ, 2ZMM and 2ZN7) bind in the primary phosphotyrosine binding site and

make several hydrogen bonds with the residues of catalytic and WPD loop (Feature 1 in Figure 5). In addition, there is a salt bridge between the 2-carboxylic acid of thiophene and Lys120 side chain (Feature 2). Phenyl ring having phosphonate moiety or thiophene ring of the ligands are located in a hydrophobic pocket and were involved in π–π stacking interactions with Tyr46 (YRD loop), Phe182 (WPD loop) (Feature 3). The next phenyl ring makes hydrophobic contacts with aliphatic side chains of Val49 and Ile219 (Feature 4).

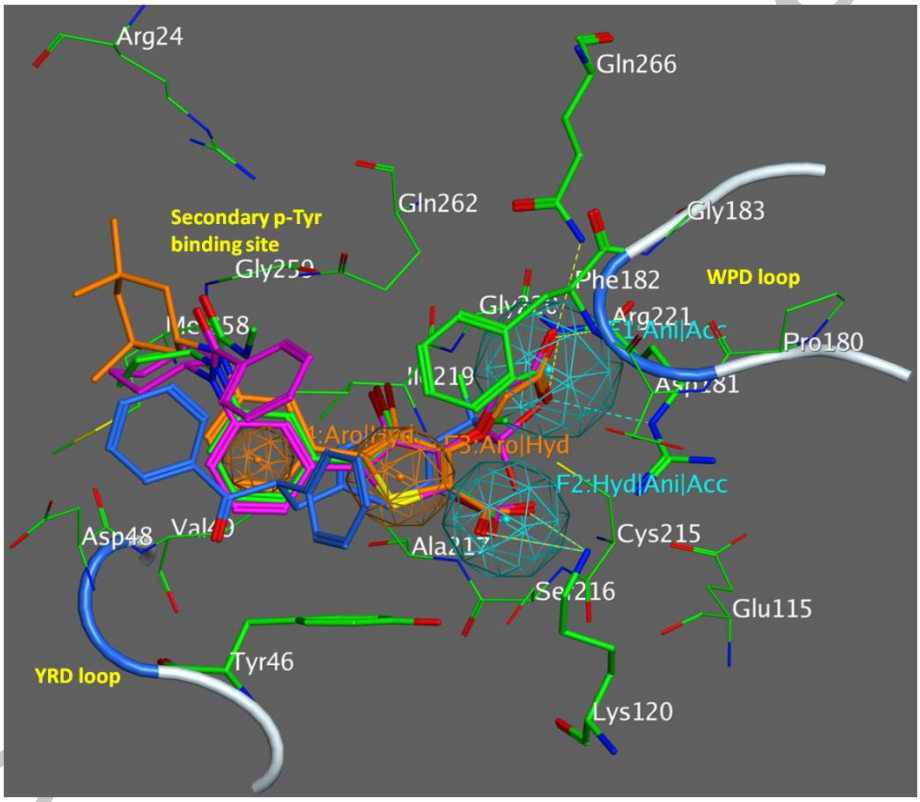


Figure 5. Structure-based pharmacophore model of PTP1B inhibitors (2QBQ-Orange; 2ZMM-Green; 2ZN7-Magenta; 3CWE-Blue color). Abbreviations: Ani (Anionic), Acc (Acceptor), Aro (Aromatic), Hyd (Hydrophobic).

The mapping of representative compounds **8j**, **11f** and **16c** on the developed pharmacophore model revealed their important functional and structural interactions with the PTP1B active site residues (Figure 6). The carboxylic acid of pyrazole maps to Features 1 and 2 (interacting with

catalytic & WPD loop residues), the pyrazole ring and the attached phenyl ring map to Features 3 and 4 respectively (π-π and hydrophobic interactions). The alignment of ligands **8j**, **11f** and **16c** is also shown in pharmacophore model (Figure 6), and the analysis showed that these compounds fit well onto the pharmacophore model in the same manner as potent co-crystallized PTP1B inhibitors. To complement the above structure-based pharmacophore studies and as an additional validation of the binding mode predictions of the designed ligands, molecular docking was performed using AutoDock Vina.[11] The binding modes of the compounds and details are depicted in Figure S1 (Supplementary Information).

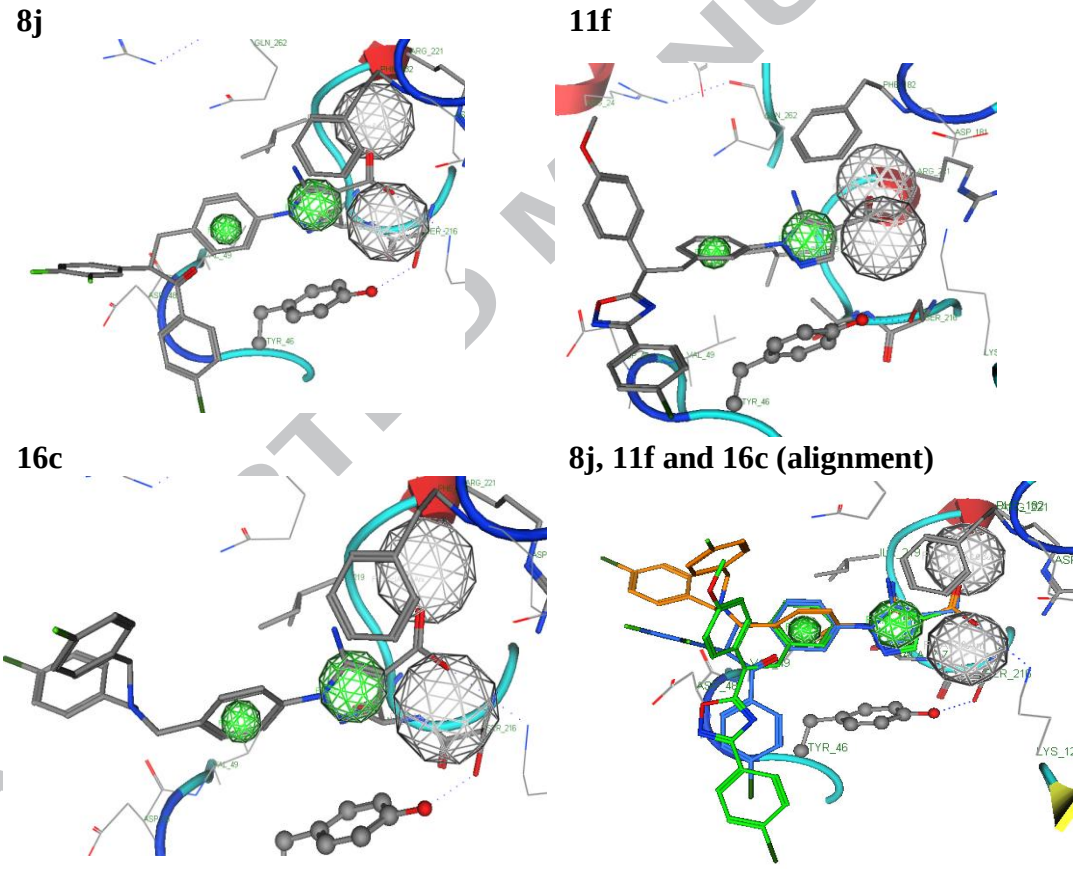
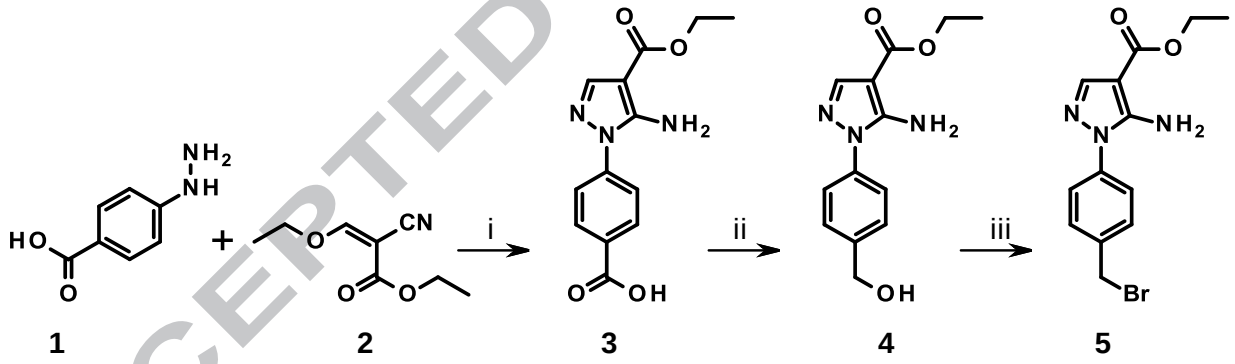


Figure 6. Overlay of **8j**, **11f** and **16c** on pharmacophore model and their alignment (**8j**-blue, **11f**-green, **16c**-orange).

3. Chemistry

In our previous article[6] we have reported the discovery of isoxazole carboxylic acid-based potent, selective and orally bio-available PTP1B inhibitors. Herein, our aim was to discover novel "5-Amino-1H-pyrazole-4-carboxylic acid" as pharmacophore which is connected to an aromatic ring (phenyl) which in turn connected to a hydrophobic tail group through a methylene or alkyl amine group. 1,2-diphenyl ethanone, benzylphenyl-1,2,4-oxadiazole and dibenzyl amine group has been selected as hydrophobic tail for the reason that these hydrophobic tail being used extensively in PTP1B drug discovery research. With this background, compounds **8a-j**, **11a-f**, and **16a-i** have been synthesized.

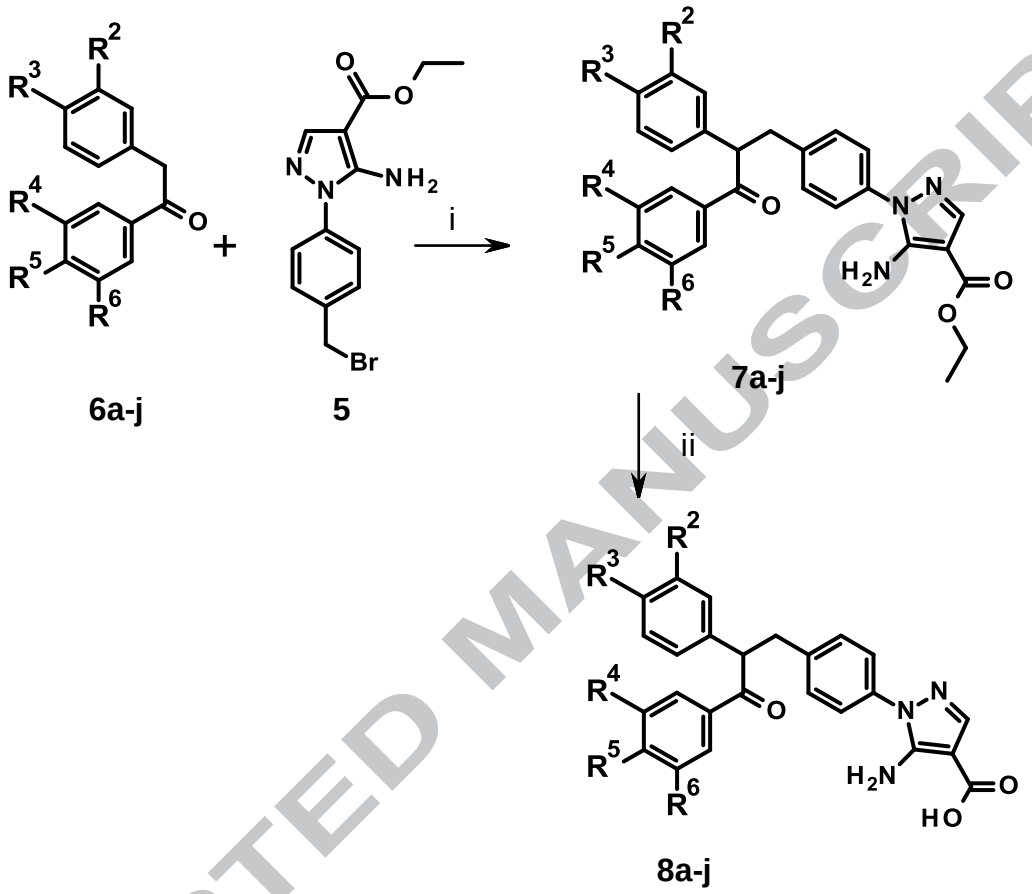
Synthesis of final compounds **8a-j**, **11a-f**, **16a-i** and their analogs are illustrated in Schemes 1-4 below.



Scheme 1. Reagents and conditions: (i) Ethanol, 90 ^{o}C, 48 h, 84%; (ii) BH_3.THF solution (1M), rt; 68%, (iii) PBr_3, DCM, 10-15 ^{o}C, 84%.

In scheme 1, compound **3** formation was carried out by reaction of **1** with **2** in ethanol at refluxed temperature. Compound **3** was then treated with borane-THF in THF at rt to obtain

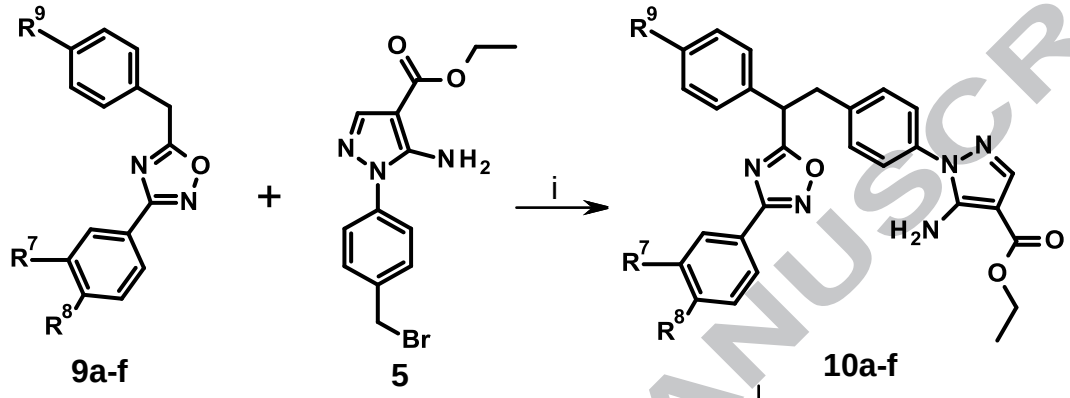
corresponding alcohol **4**. It was then brominated using PBr_3 in DCM at temperature 0-5 ^oC to obtain compound **5** in good yield.



Scheme 2. Reagents and conditions: (i) NaH, DMF, 10-15 ^oC, 3 h, 61-98%; (ii) $LiOH.H_2O$, dioxane: H_2O (4:1), 40 ^oC, 16 h, 55-75%.

Synthetic route to compounds **8a–j** is outlined in Scheme 2. Key benzyl bromide **5** thus synthesized was treated with ethanone derivatives[6] **6a-j** in presence of NaH in DMF at temperature10-15 ^oC to obtain **7a-j** which on hydrolyzed with $LiOH.H_2O$ at 40 ^oC provided target compound **8a-j** (Table 2) in overall good yields (55-75%).

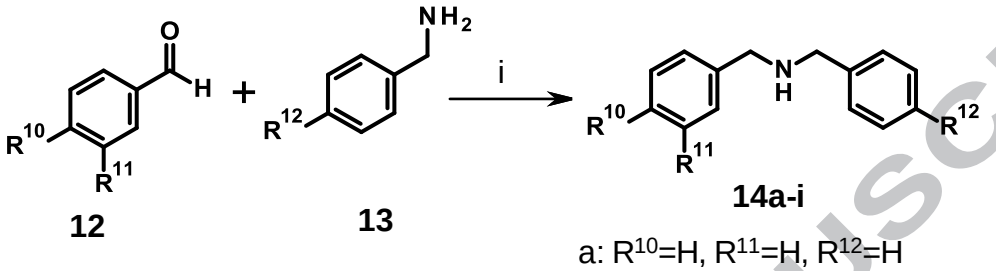
As outlined in scheme 3, oxadiazole derivatives[6] **9a-f** were treated with **5** in presence of NaH in DMF at temperature 10-15 ^{o}C yielded corresponding ester derivatives (**10a-f**) which on hydrolyzed with LiOH.H_2O at rt provided target compounds **11a-f** in overall good yields (60-80%).



Scheme 3. Reagents and conditions: (i) NaH, DMF, 10-15 ^{o}C, 3 h, 55-85%; (ii) LiOH.H_2O, dioxane: H_2O (4:1), 40 ^{o}C, 16 h, 60-80%.

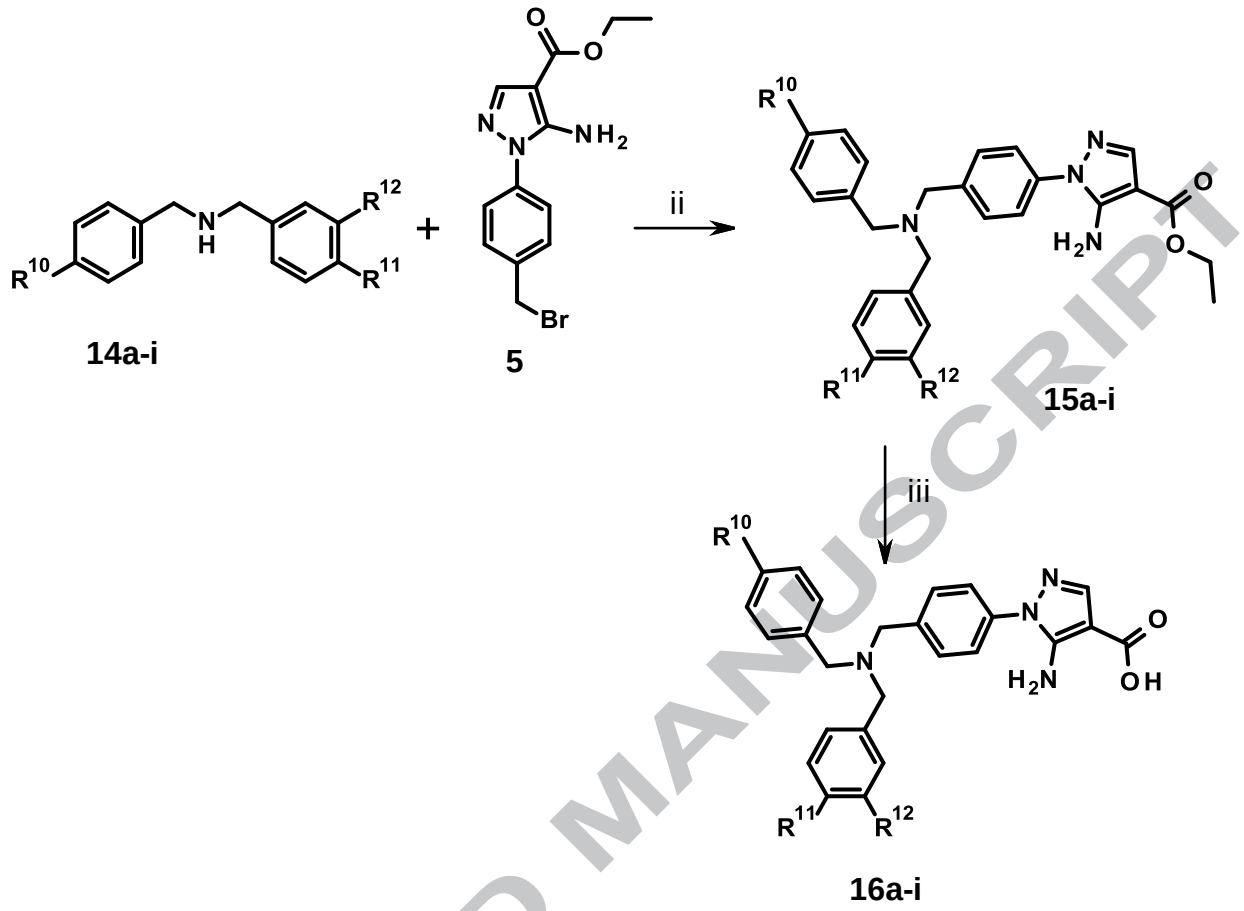
The synthesis of compounds **16a–i** is outlined in Scheme 4. Dibenzyl amine derivatives (**14a-i**) were prepared by reaction of aldehyde and benzyl amine in dichloroethane in presence of sodium

triacetoxyborohydride and catalytic amount of acetic acid.[12] Dibenzyl amine derivatives (**14a-i**) were then treated with key benzyl bromide (**5**) in presence of Cs_2CO_3 in DMF at 20-25 ^oC to obtain ester **15a-i** which on hydrolyzed with $LiOH.H_2O$ provided target compounds **16a-i** in good overall yields (50-95%).



12 **13** **14a-i**

a: R^{10}=H, R^{11}=H, R^{12}=H
b: R^{10}=F, R^{11}=F, R^{12}=H
c: R^{10}=F, R^{11}=F, R^{12}=F
d: R^{10}=F, R^{11}=Cl, R^{12}=H
e: R^{10}=F, R^{11}=OMe, R^{12}=H
f: R^{10}=F, R^{11}=OMe, R^{12}=OMe
g: R^{10}=Cl, R^{11}=F, R^{12}=F
h: R^{10}=OMe, R^{11}=OMe, R^{12}=H
i: R^{10}=Me, R^{11}=OMe, R^{12}=H

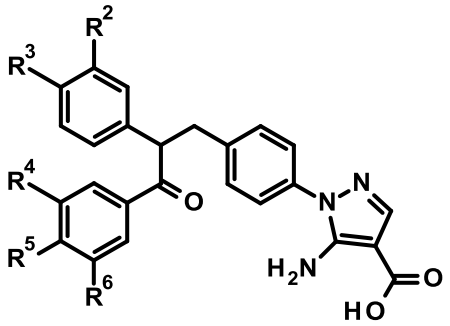


Scheme 4. Reagents and conditions: (i) $Na(OAc)_3BH$, acetic acid, DCE, 20-25 ^oC, 12 h, 43-82%; (ii) Cs_2CO_3, DMF, 10-15 ^oC, 16 h, 60-85%; (iii) $LiOH.H_2O$, dioxane: H_2O (4:1), 40 ^oC, 16 h, 50-90%.

4. Results and discussion

All the derivatives (**8a-j**, **11a-f** and **16a–i**) were evaluated in fluorescence based kinetic assay as per the procedure described in our earlier publication.[6]

Table 2. Investigation of 1,2-diphenyl ethanone analog as hydrophobic tail.

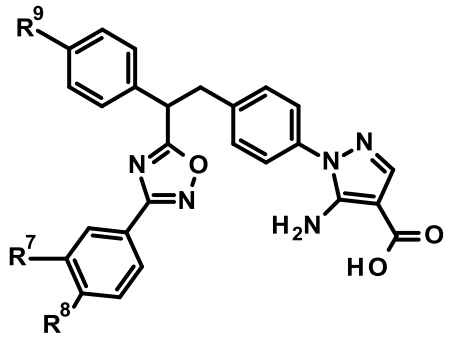


Compd.	R_2	R_3	R_4	R_5	R_6	% PTP1B Inhibition at	
						10 μM	100 μM
8a	H	H	H	H	H	9.6 ± 2.1	32.6 ± 1.1
8b	H	F	H	H	H	19.0 ± 3.3	43.9 ± 1.5
8c	H	H	H	F	H	12.1 ± 4.5	36.8 ± 1.4
8d	H	F	H	F	H	0.8 ± 0.7	34.4 ± 2.2
8e	H	F	H	OMe	H	13.5 ± 0.8	58.5 ± 1.9
8f	H	OMe	H	OMe	H	4.8 ± 0.9	39.7 ± 4.8
8g	H	F	H	Cl	H	18.8 ± 3.4	89.5 ± 0.8
8h	F	F	H	H	H	10.5 ± 0.3	48.5 ± 1.1
8i	F	F	H	F	H	21.6 ± 0.4	82.2 ± 2.3
8j	F	F	H	Cl	H	16.2 ± 3.6	91.5 ± 0.7

Simple unsubstituted compound **8a** showed poor activity against PTP1B enzyme assay. To explore SAR, small hydrophobic group like –F was incorporated in the phenyl ring (R^3 or R^5), but it also did not improve activity (**8b-c**). A variety of other di-*para*-substituents were also significantly less active (**8d-f)**. Further optimization revealed that incorporation of *para*-chloro

(R^3=Cl) in compound **8c** yielded **8g** which improves potency. Addition of *meta*-fluoro (R^2=F) substituents in compound **8d** and **8g** was tolerated and gave **8i** and **8j** which are equipotent to **8g**. The most potent compounds **8i** and **8j** were found to be metabolically stable in both mouse (CLintr: <1 mL/min/g liver) and rat liver CLintr: <2 mL/min/g liver) microsomes.[6] Our initial SAR was focused on 1,2-diphenyl ethanone containing compounds (Table 2). Then we intended to synthesize compounds **11a-f** by replacing keto group of 1,2-diphenyl ethanone with more rigid oxadiazole, which further yielded more potent derivatives (Table 3). Unsubstituted compound **11a** was found to be inferior to 11**b** (R^8=R^9=F). Attempt was made to improve potency by introducing different substituents on tail phenyl ring in compound **11b**. Small hydrophobic substitutions like F, Cl and hydrophilic substitution like -OMe (examples **11c-f**) yielded equipotent compounds like **11b**. In general *para*-substituents are more active than *meta*-substituents. To understand the metabolic stability of this series compounds **11c-f** were tested in rodent liver microsomes[6] and these were found to be metabolically stable as these compounds have low intrinsic clearance.

Table 3. Investigation of oxadiazole analogs as hydrophobic tails.



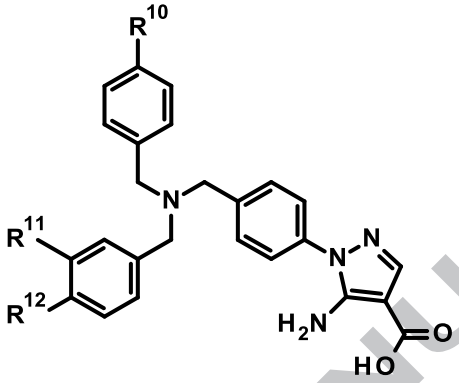
Compd.	R_7	R_8	R_9	% PTP1B Inhibition at		K_i (μM)	CL_{intr}[a] (mL/min/g liver)	
				10 μM	100 μM		MLM	RLM
11a	H	H	H	12.2 ± 0.5	64.0 ± 2.3	-	-	-
11b	F	H	F	19.0 ± 0.4	94.9 ± 0.3	-	-	-
11c	H	Cl	F	30.8 ± 1.9	97.3 ± 0.2	4.6	0.0	3
11d	H	OMe	F	25.8 ± 1.8	95.5 ± 0.2	8.1	1.5	3
11e	F	H	OMe	22 ± 1.0	93.3 ± 0.4	8.6	1.3	3
11f	H	Cl	OMe	33.7 ± 0.5	98.3 ± 0.2	4.4	1.5	2

[a]Intrinsic clearance < 2 mL/min/g liver or 80% remaining at 30 min is considered as metabolically stable. MLM: Mouse liver microsomes, RLM: Rat liver microsomes.

We next turned out our attention to the di-benzyl analogs (Table 4), since these compounds contain *tert*-nitrogen, it is expected to improve cell permeability. Simple unsubstituted compound **16a** showed better potency than corresponding ethanone and oxadiazole compound. Attempt was made to improve activity further by introducing *para*-substituents and small hydrophobic groups like –F, -Cl, -OMe were found to be tolerated. We found that introduction of –Cl improves potency and **16c** showed good inhibitory activity in PTP1B enzymatic assay with

K_i value of 4.6 μM. Replacing -Cl with –OMe in compound **16c** yielded **16d** which looses potency, whereas other disubstituted compounds **16e-f** significantly losses potency.

Table 4. Investigation of dibenzylamine analogs as hydrophobic tail.



Compd.	R_{10}	R_{11}	R_{12}	% PTP1B Inhibition at		K_i (μM)	CL_{intr} (mL/min/g liver)	
				10 μM	100 μM		MLM	RLM
16a	H	H	H	8.9 ± 2.0	64.0 ± 2.3	-	-	-
16b	F	H	F	40.8 ± 1.2	98.4 ± 0.7	-	-	-
16c	F	H	Cl	43.8 ± 2.6	97.4 ± 0.9	4.5	2.1	3.0
16d	F	H	OMe	21.6 ± 0.4	82.2 ± 2.3	-	-	-
16e	OMe	H	Me	5.0 ± 1.0	49.7 ± 0.7	-	-	-
16f	OMe	H	OMe	9.9 ± 2.9	76.9 ± 1.3	7.1	5.6	2.5
16g	F	F	F	19.5 ± 1.2	95.2 ± 1.1	13	2.7	3.0
16h	Cl	F	F	22.5 ± 3.2	97.2 ± 1.9	11	1.2	2.0
16i	F	OMe	OMe	35.07 ± 0.8	94 ± 0.2	4.1	7.4	4.0

Addition of one more –F, -Cl yielded compound **16g-h**, which showed better potency. Incorporation of -OMe in **16d** yielded equipotent compound **16i**. This series have shown moderate to good stability in rodent liver microsomes.

Based on our hypothesis to improve the cell permeability by increasing the pka of the compounds, we have evaluated a few compounds in parallel artificial membrane permeation assay (PAMPA) at physiological pH (Table 5). Ethanone derivative (**8j**) showed moderate intrinsic permeability and oxadiazole derivatives (**11c-d**) showed good to low intrinsic permeability. However, dibenzyl derivatives (**70c**, **70f-i**) showed excellent intrinsic permeability in PAMPA. Furosemide and propanol were used as a positive control in this assay.

Table 5. PAMPA permeability values for a few synthesized compounds.

Compound	Permeability in PAMPA at pH 7.4 (nm/sec)
8j	15.0
11c	91.0
11d	4.0
16c	106.0
16f	125.0
16g	278.9
16h	498.0
Furosemide	< 1
Propanolol	166

5. Conclusion

In summary, a series of "amino-carboxylic based pyrazole" was discovered as novel pharmacophore by structure-based pharmacophore model and molecular docking analysis. In comparison to most of the existing PTP1B inhibitors, our designed molecules contain small molecular weight, non-phosphonate head groups. Functional groups such as 1,2-diphenyl ethanone, oxdiadizole and dibenzyl amines were selected as hydrophobic tail. A series of compounds containing these hydrophobic tails were synthesized and evaluated in PTP1B enzymatic assay. In general, oxadiazole derivatives (**11a-f**) and dibenzyl amines (**16a-i)** were more potent as compared ethanone (**8a-j**) derivatives. These compounds were found to be stable in rodent liver microsomes. Dibenzyl amine derivatives were found to be better cell permeable in PAMPA than ethanone and oxadiazole derivatives. It is assumed that these PTP1B inhibitors will act as a lead to identify more potent compounds with good cell permeability and oral bioavailability for the treatment of Type 2 Diabetes.

6. Experimental section

6.1. General synthetic methods

The purity of compounds was determined by HPLC. 1H nuclear magnetic resonance (NMR) spectra were recorded in the deuterated solvents specified on a Varian 400 spectrometer operating at 400. The signal of the deuterated solvent was used as internal reference. Chemical shifts (δ) are given in ppm and are referenced to residual not fully deuterated solvent signal. Coupling constants (J) are given in Hz. Chemical shifts are reported in parts per million (δ) from the tetramethylsilane resonance in the indicated solvent (TMS: 0.0 ppm). Data are reported as follows: chemical shift, multiplicity (br=broad, s=singlet, d=doublet, t=triplet, q=quartet,

m=multiplet) and integration. Mass spectra were determined by using Agilent 1200SL-6110 LC/MS (ESI) system using positive-negative switching.

6.1.1. General procedure for the preparation of 7a-j

To an ice-cold suspension of NaH (1.2 mol equivalent) in DMF (5 fold), a solution of **6a-j** (1.0 to 1.4 mol equivalent) in DMF (3 fold) was added drop wise over a period of 10 minutes and stirred at the same temperature for 30 minutes. A solution of **5** (1.0 mol equivalent) in DMF (2 fold) was added to the reaction mixture at 20-25 ℃ and stirred at that temperature for 2 h. The reaction mixture was poured into water and aqueous layer was extracted with ethyl acetate (2 X 10 mL). The combined organic layer was successively washed with water and brine, dried over Na2SO4, filtered and concentrated under vacuum. The crude product was purified by column chromatography using 5-10% ethyl acetate in hexane as an eluent to obtain title compound (**7a-j**).

Ethyl 5-amino-1-[4-[3-(4-chlorophenyl)-2-(3,4-difluorophenyl)-3-oxo-propyl]phenyl]pyrazole-4-carboxylate (**7j**)

7j (0.156 g, 100%) was prepared from **6j** (0.097 g, 0.36 mmol) and **5** (0.1 g, 0.30 mmol) following the general procedure described above as colorless mass.

^{1}HNMR (DMSO d_6): δ 1.26 (t, *J* = 7.2 Hz, 3H), 3.09 (dd, *J* = 7.4, 13.8 Hz, 1H), 3.48 (dd, *J* = 7.4, 13.8 Hz, 1H), 4.21 (q, *J* = 6.8 Hz, 2H), 5.36 (t, *J* = 7.2 Hz, 1H), 6.25 (br s, 2H), 7.19 (br s, 1H), 7.31-7.38 (m, 5H), 7.47-7.49 (m, 1H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.67 (s, 1H), 8.07 (d, *J* = 8.4 Hz, 2H); ESI/MS (m/z): 510.1 $(M+H)^+$

6.1.2. General procedure for the preparation of the compounds 8a-j

To a solution of **7a-j** (1 mol equivalent) in dioxane:H_2O (4:1) (10 fold), was added LiOH.H_2O (3 mol equivalent) and stirred at temperature 40 ^oC for 16 h. The organic volatiles were evaporated and residue was diluted with water. The aqueous layer was washed with ethyl acetate (2 X 5 mL) and aqueous layer was cooled to 0-5 ^oC. The aqueous layer was acidified with 2(N) HCl and precipitate was separated, filtered off, washed with water and dried to furnish title product **8a-j**.

8j: 5-Amino-1-[4-[3-(4-chlorophenyl)-2-(3,4-difluorophenyl)-3-oxopropyl]phenyl]pyrazole; White solid; mp: 117-118 ^oC; Yield: 49%; Purity: 98.0% by HPLC; ^{1}HNMR (DMSO d_6): δ 3.09 (dd, *J* = 7.4, 13.8 Hz, 1H), 3.45 (dd, *J* = 7.4, 13.8 Hz,1H), 5.36 (t, *J* = 7.4 Hz, 1H), 6.21 (br s, 2H), 7.19 (br s, 1H), 7.34-7.41 (m, 5H), 7.44-7.49 (m, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.64 (s, 1H), 8.07 (d, *J* = 8.4 Hz, 2H), 12.07 (br s, 1H); ESIMS m/z: 482.1 $(M+H)^+$.

6.1.3. General procedure for the preparation of the compounds 11a-f

To a solution of **10a-f** (1 mol equivalent) in dioxane: H_2O (4:1) (10 fold), was added LiOH.H_2O (3 mol equivalent) and stirred at 40 ^oC for 16 h. The organic volatiles were evaporated and residue was diluted with water. The aqueous layer was washed with ethyl acetate (2 X 5 mL) and aqueous layer was cooled to 0-5 ^oC. The aqueous layer was acidified with 2(N) HCl and precipitate was separated, filtered off, washed with water and dried to furnish title product **11a-f.**

11c: 5-Amino-1-[4-[2-[3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl]-2-(4-fluorophenyl)ethyl]phenyl]pyrazole-4-carboxylic acid, White solid; Yield: 78%; Purity: 95.0% by HPLC; ^{1}HNMR (DMSO d_6): δ 3.45 (dd, *J* = 8.2, 13.8 Hz, 1H), 3.67 (dd, *J* = 8.0, 14.0 Hz, 1H), 5.04 (t, *J* = 8.0 Hz, 1H), 6.23 (br s, 2H), 7.19 (t, *J* = 8.8 Hz, 2H), 7.41 (br s, 4H), 7.50-7.53 (m, 2H), 7.64 (br s, 2H), 7.66 (s, 1H), 8.02 (d, *J* = 8.8 Hz, 2H), 12.08 (br s, 1H).ESIMS m/z: 504.2 $(M+H)^+$.

6.1.4. General procedure for the preparation of the compounds 16a-i

These compounds were prepared in the same manner as following the general procedure **11a-f**

16f: 5-Amino-1-[4-[[bis[(4- methoxyphenyl)methyl]amino]methyl]phenyl]pyrazole-4-carboxylic acid; White solid; mp: 172-174 ^{o}C; Yield: 52%; Purity: 99.0% by HPLC; ^{1}HNMR (DMSO d_6): δ 3.49 (s, 4H), 3.55 (s, 2H), 3.77 (s, 6H), 6.29 (br s, 2H), 6.95 (d, *J* = 7.6 Hz, 4H), 7.34 (d, *J* = 8.0 Hz, 4H), 7.55 (br s, 4H), 7.69 (s, 1H), 12.06 (br s, 1H); ESIMS m/z: 473.2 $(M+H)^{+}$. **16g:** 5-**Amino-1-[4-[[(3, 4-difluorophenyl)methyl-[(4-fluorophenyl)methyl]amino]methyl]phenyl]pyrazole-4-carboxylic acid**; White solid; mp: 168-169 ^{o}C; Yield: 67%; Purity: 95.0% by HPLC; ^{1}HNMR (DMSO d_6): δ 3.53 (s, 4H), 3.56 (s, 2H), 6.27 (br s, 2H), 7.18 (t, J = 8.8 Hz, 2H), 7.26 (br s, 1H), 7.39-7.45 (m, 4H), 7.50-7.54 (m, 4H), 7.66 (s, 1H), 12.07 (br s, 1H); ESIMS m/z: 467.2 $(M+H)^{+}$. **16h: 5-Amino-1-[4-[[(4-chlorophenyl)methyl-[(3,4-difluorophenyl)methyl]amino]methyl]phenyl]pyrazole-4-carboxylic acid;** White solid; mp: 166-170 ^{o}C; Yield: 53%; Purity: 98.0% by HPLC; ^{1}HNMR (DMSO d_6): δ 3.53 (s, 4H), 3.56 (s, 2H), 6.26 (br s, 2H), 7.18 (t, *J* = 8.8 Hz, 2H), 7.20-7.25 (m, 1H), 7.39-7.45 (m, 4H), 7.50-7.54 (m, 4H), 7.66 (s, 1H), 12.05 (br s, 1H); ESIMS m/z: 483.0

Acknowledgements

Authors are grateful to Drs. Kasim A. Mookhtiar and Venkata P. Palle for their support, management of Advinus Group for encouragement. Analytical departments are being acknowledged for their help during this work. We thank Dr. Anup Ranade for managing intellectual property. Advinus publication no. ADV-A-035

Supplementary Material

Molecular Docking analysis, Brief of PAMPA screening assay protocol and experimental procedure.

References and notes:

1. Tonks, N. K. *FEBS Letters*. **2003**, *546*, 140.
2. (a) Saltiel, A. R.; Kahn, C. R. *Nature.* **2001**, 414, 799*;* (b) Evans, J. L.; Jallal, B. *Expert Opin. Investig. Drugs.* **1999**, 8, 139
3. (a) Cheng, A.; Dube, N.; Gu, F.; Tremblay, M. L. *Eur. J. Biochem*. **2002**, *269*, 1050. (b) Zhang, Z.; Lee, S. Y. *Expert Opin. Investig. Drugs* **2003**, *12*, 223.
4. (a) Dufresne, C.; Roy, P.; Wang, Z.; Asante-Appiah, E.; Cromlish, W.; Boie, Y.; Forghani, F.; Desmarais, S.; Wang, Q.; Skorey, K.; Waddleton, D.; Ramachandran, C.; Kennedy, B. P.; Xu, L.; Gordon, R.; Chan, C. C.; Leblanc, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1039; (b) Lau, C. K.; Bayly, C. I.; Gauthier, J. Y.; Li, C. S.; Therien, M.; Asante-Appiah, E.; Cromlish, W.; Boie, Y.; Forghani,F.; Desmarais, S.; Wang, Q.; Skorey, K.; Waddleton, D.; Payette, P.; Ramachandran, C.; Kennedy, B. P.; Scapin,G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1043.
5. (a) Liu, G.; Trevillyan, J. M. *Curr. Opin. Investig. Drugs* **2002**, *3*, 1608; (b) Johnson, T. O.; Ermolieff, J.; Jirousek, M. R. *Nat. Rev. Drug Discov.* **2002**, *1*, 696; (c) Andersen, H. S.; Iversen, L. F.; Jeppesen, C. B.; Branner, S.; Norris, K.; Rasmussen, H. B.; Moller, K. B. *J. Biol. Chem.* **2000**, *275*, 7101; (d) Andersen, H. S.; Olsen, O. H.; Iversen, L. F.; Sørensen, A. L. P.; Mortensen, S. B.; Christensen, M. S.; Branner, S.; Hansen, T. K.; Lau, J. F.; Jeppesen, L.; Moran, E. J.; Su, J.; Bakir, F.; Judge, L.; Shahbaz, M.; Collins,

T.; Vo, T.; Newman, M. J.; Ripka, W. C.; Møller, N. P. H. *J. Med. Chem.* **2002**, *45*, 4443. (d) Haftchenary, S: Jouk, O. A; Aubry, I, Lewis, M. A; Landry, M; Ball, P. D; Shouksmith, E. A; Collins, V. C; Tremblay, L. M; Gunning, T. *ACS Med. Chem. Lett*. **2015**, *6*, 982

6. Basu, S.; Prasad, V. U.; Barawkar, D; De, S.; Menon, S; Venkata, P.; Meena, P.; .Sachin, T.; Umesh, P.; S; Sarma, K.; Waman. Y.; Niranjan, S.; Pathade, V.; Gaur, A.; Reddy, S.; Ansari, S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2843.
7. (a) Combs, A. P. *IDrugs* **2007**, *10*, 112.; (b) Combs, A. P. *J. Med. Chem*. **2010**, *53*, 2333.; (c) Bhattarai, B. R.; Kafle, B.; Hwang, Ji-Sun.; Ham, S. W.; Lee, K.H.; Park, H.; Han, I.; Cho, H. *Bioorg. Med. Chem. Lett*. **2010**, *20*, 6758.; (d) Meanwell N. A. J. Med. Chem. **2011**, *54*, 2529.; (e) Zhi, Y.;Gao, Li-Xin.; Jin, Y.; Tang, Chun-Lan.; Li, Jing-Ya.; Long, Ya-Qiu. *Bioorg. Med. Chem. Lett*. **2014**, *22*, 3670. (f) Zhang, L.; Jiang, Cheng-Shi.; Gao, Li-Xin.; *Bioorg. Med. Chem. Lett*. **2016**, *26*, 778.; (g) Abdjul, D.B.; Yamazaki, H.; Takahashi, O.; Ukai, K.; Namikoshi, M.; *Chem. Pharm. Bull*. **2016**, *64*, 733.
8. Gong, Jing-Xu.; Wang, Zhong-Hua.; Li, Jing-Ya.; Li, J; Li, Xu-Wen.; Guo, Yue-Wei. *Bioorg. Med. Chem. Lett*. 2010, 20, 6758
9. Liu,G.; Xin, Z.; Pei, Z.; Hajduk, P. J.; Abad-Zapatero, C.; Hutchins, C. W.; Zhao, H.; Lubben, T. H.; Ballaron, S. J.; Haasch, D. L.; Kaszubska, W.; Rondinone, C. M.; Trevillyan, J. M.; Jirousek, M. R. *J. Med. Chem.* **2003**, *46*, 4232.
10. Zhao; H.; Liu,G.; Xin, Z.; Serby, M.; Pei, Z.; Szczepankiewicz, B.; Hajduk, J.; Abad-Zapatero, C.;Hutchins, C.; Lubben, H.; Ballaron, S.; Haasch, D.; Kaszubska, W.; Rondinone, C.; Trevillyana, J.; Jirouseka, M.R. *Bioorg. Med. Chem. Lett*. **2004**, *22*, 5543

11. O. Trott, A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, *Journal of* Computational Chemistry **2010**, 31,455.

12. Abdel-Magid, A.F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R.D.Bioorg.; *J. Org. Chem*. **1996**, *61*, 3849.